New Plant Regulators Based on 2-Hydroxycyclohexyl Quaternary Ammonium Compounds

William F. Newhall

Dodecyl-(2-hydroxycyclohexyl)dimethylammonium bromide and similar compounds represent a new class of plant regulators synthesized by reaction of cyclohexene oxide with dimethylamine and quaternization of the resulting amino alcohol with various alkyl halides. These compounds demonstrate a high degree of growth regulant activity in bioassays on alfalfa seed (*Medicago sativa* L., Hairy Peruvian) and cucumber seed (*Cucumis sativus* L., Marketer). When applied in lanolin to young bean plants (*Phaseolus vulgaris* L., Black Valentine), they cause marked internode shortening, darker green leaf color, and overall height reduction without causing any formative changes. Five of these compounds are more active growth regulators than succinic acid 2,2-dimethylhydrazide (SADH). Foliar sprays of two of these derivatives applied separately on young grapefruit seedlings (*Citrus paradisi* Macf.) at 3000 ppm in water strongly retarded vegetative growth for 3 weeks.

The biological activity of quaternary ammonium salts as fungicides, insecticides, and bactericides has been reported extensively in the literature (De Benneville, 1956). Quaternary ammonium compounds have also been shown to be active plant growth regulators (Knight *et al.*, 1969), the best known being (2-chloroethyl)trimethylammonium chloride (chlormequat) (Tolbert, 1959) and 4-hydroxy-5-isopropyl-2-methylphenyltrimethylammonium chloride 1-piperidine carboxylate (AMO-1618) (Krewson *et al.*, 1959). The synthesis, from the terpene (+)-limonene, of quaternary salts which are growth regulators on a number of plants including citrus has been reported (Newhall, 1971, 1972); Newhall and Pieringer, 1966, 1969; Pieringer and Newhall, 1968).

Studies on the relationship between chemical structure and plant regulator activity, in order to discover highly active molecules less complex than those now in use, have led to the synthesis of nonterpenoid 2-hydroxycyclohexylammonium compounds which are the subject of this paper. Several authors have reported the synthesis of quaternary ammonium salts containing a cyclohexyl moiety and discussed their activity as bactericides (Winternitz *et al.*, 1951). However, none of these articles suggested the high order of plant regulator activity observed with the simplest 2-hydroxycyclohexylammonium compounds.

METHODS AND PROCEDURES

Compound Preparation. The starting material used for the preparation of all these quaternary ammonium compounds was 2-dimethylamino-1-cyclohexanol. Its preparation by the reaction of cyclohexene oxide with dimethylamine has been reported (Shapiro *et al.*, 1959). Each quaternary ammonium compound was synthesized by the reaction of 2-dimethylamino-1-cyclohexanol (2.5 g) with an excess (6.0 g) of the appropriate alkyl bromide in solution in 2.5 ml of dimethylformamide for 16 hr at 98°. The yield of product from each quaternization was 100% of theoretical. These compounds were characterized by elemental nitrogen analyses (Table I) and infrared absorption spectra which were consistent in all cases with the assigned structures. The general structure for this new class of plant regulators is also presented in Table I. Ten derivatives were prepared in which the length of the *n*-alkyl chain (R) was 7–16 carbon atoms. Only the hexadecyl compound has been synthesized previously (Winternitz *et al.*, 1951) for use as a bactericide.

University of Florida, IFAS, Agricultural Research and Education Center, Lake Alfred, Florida 33850.

Growth Regulator Tests. Three bioassays were employed to determine the regulant activities of these ten compounds. A bioassay based on the radicle growth of cucumber Marketer seed (Mitchell and Livingston, 1968) was particularly sensitive to these compounds in the concentration range 0.0005–0.00002 *M*. Three replicates of ten seeds each were used for each of the five concentrations of test compound employed (0.0005, 0.0002, 0.0001, 0.00005, and 0.00002 *M*). Seeds were placed on filter paper in 9-cm petri dishes conaining 4 ml of solution and kept in the dark at 25° for 4 days. Radicles were then measured and the molar concentration giving 50% growth retardation (I_{50}) was determined from a semilog plot of concentration against per cent retardation. The plots were linear in every case. These I_{50} concentrations were plotted against the number of carbons in the alkyl group R attached to nitrogen.

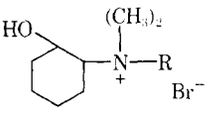
A bioassay (R. H. Biggs, 1973) based on the growth response of Hairy Peruvian alfalfa seedlings was also used to determine growth retardant activity. For this test, three replicates of 100 seeds each were used for each of the five concentrations of test compound employed (0.005, 0.002, 0.001, 0.0005, and 0.0002 *M*). Seeds were placed on filter paper in 6-cm petri dishes containing 2 ml of test solution and kept in the dark at 25° for 24 hr. The seeds were then exposed to light from cool white fluorescent lamps for an additional 24 hr. Excess solution was removed by gently pressing the plants between filter paper and the seedlings were weighed to 0.1 mg. Retardant activity was calculated as per cent weight reduction in comparison with untreated controls. Molar I_{50} values were determined graphically as for cucumber. All such plots were linear. These I_{50} concentrations were plotted against the number of carbons in the alkyl group R attached to nitrogen.

For testing on Black Valentine bean plants, 1% concentrations of test compounds and SADH were prepared in lanolin containing 2.5% Tween 80 (Krewson *et al.*, 1959). A 15–20-mg ring of these pastes was applied to the first internode of plants when the primary leaves were fully expanded and the trifoliate leaves were still tightly folded in the terminal buds. Plant height and length of second internode were measured after 7 days' growth in the greenhouse. Each treatment comprised six replicates and the entire experiment was performed three times. Results were expressed as the average per cent reduction in growth as compared to control plants treated with lanolin containing 2.5% Tween 80.

Foliar Sprays on Grapefruit Seedlings. Grapefruit seedlings, grown in the greenhouse for approximately 1 year, were selected for uniform height (40–45 cm). The plants were cut back to 25 cm above the can top and

Table I. Quaternary Ammonium Derivatives of 2-Dimethylaminocyclohexanol

General formula



R	% N Calcd	% N Found[a]
Heptyl	4.34	4.56
Octyl	4.16	4.29
Nonyl	3.99	4.19
Decyl	3.84	3.97
Undecyl	3.70	3.90
Dodecyl	3.56	3.84
Tridecyl	3.44	3.68
Tetradecyl	3.33	3.47
Pentadecyl	3.22	3.24
Hexadecyl	3.12	3.16

[a] Nitrogen analyses were made using a semimicro-Kjeldahl–Gunning procedure. This was necessary because only the hexadecyl compound was crystalline, all others being hygroscopic glasses which were difficult to purify, dry, and weigh.

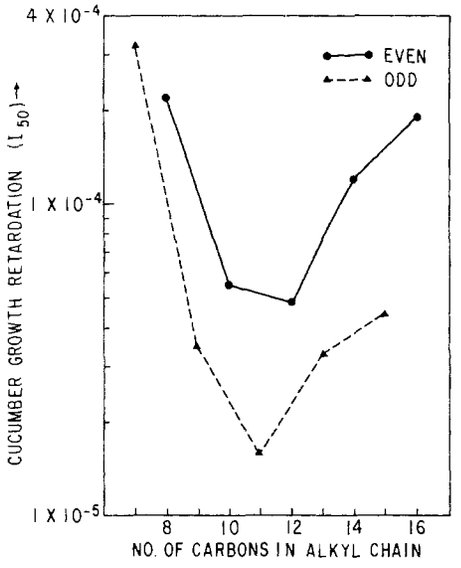


Figure 1. Effect of number of carbons in the *n*-alkyl group R (Table I) on response of cucumber radicles to quaternary ammonium compounds; I_{50} = concentration required for 50% inhibition of growth.

sprays applied 3 weeks later when new growth was well developed. One per cent of the adjuvant Regulaid (Colloidal Products) was incorporated in each spray treatment. Five compounds (Table I, R = octyl, decyl, dodecyl, tetradecyl, hexadecyl) were applied at a concentration of 3000 ppm to run off. A cover was used to prevent spray droplets from wetting the soil. Each compound and a Regulaid control were applied to ten previously measured seedlings. Plants were maintained in the greenhouse and height measurements made at weekly intervals. Any lateral shoots were removed during the course of the experiment and only the terminal bud was allowed to develop. Results were expressed as per cent activity, *i.e.*, average per cent reduction in growth as compared to the controls. The data were subjected to analysis of variance, and the mean differences were compared by the Duncan multiple range test (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

The cucumber bioassay data (Figure 1) are presented in two separate activity plots. These show the effect of the number of carbons in the *n*-alkyl group R (Table I) on the response of cucumber to quaternary ammonium compounds. The solid line shows the activities of the derivatives in which the alkyl group R has an even number of carbons and the dotted line shows the activities of those having an odd number of carbons. For each series, there is a narrow zone of maximum activity between C_{10} and C_{11}. The odd carbon chain derivatives are generally more active than those having an even number of carbons in the chain, C_{11} being by far the most active growth retardant (Figure 1).

Table II. Growth Retardant Activity of Quaternary Ammonium Derivatives of 2-Dimethylaminocyclohexanol on Black Valentine Beans

Compd	Av % reduction[a] 2nd internode	Av % reduction[a] Height
SADH	68.9cd[c]	40.2bcd
Heptyl[b]	83.5ab	50.7a
Octyl	88.4a	53.7a
Nonyl	88.7a	53.7a
Decyl	79.8abc	50.3ab
Undecyl	73.5bc	47.3ab
Dodecyl	54.8ef	39.7cde
Tridecyl	56.9de	42.5bc
Tetradecyl	43.5fg	35.2cde
Pentadecyl	38.4gh	33.2de
Hexadecyl	31.2h	29.8de

[a] Average per cent reduction compared with controls. [b] R attached to nitrogen (Table I). [c] Mean separation, within columns by Duncan's multiple range test at the 5% level.

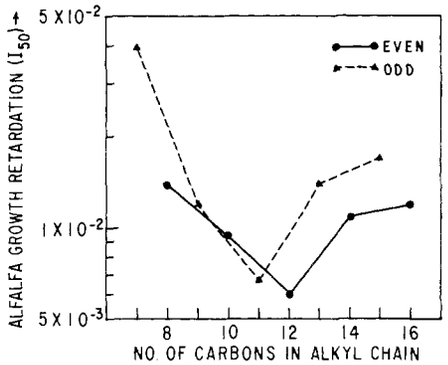


Figure 2. Effect of number of carbons in the *n*-alkyl group R (Table I) on response of alfalfa plants to quaternary ammonium compounds; I_{50} = concentration required for 50% inhibition of growth.

Figure 2 shows the effect of number of carbons in the *n*-alkyl group R (Table I) on the response of alfalfa plants to quaternary ammonium compounds. In the alfalfa bioassay as well, each series has a narrow zone of maximum activity at C_{11} for the odds and at C_{12} for the evens (Figure 2). However, in this bioassay, the two curves are close together and the even carbon chain compounds are only slightly more active than the odds. The C_{12} compound is the most active retardant in this test.

On Black Valentine beans, the heptyl, octyl, nonyl, decyl, and undecyl derivatives were more active in both internode and height reduction than SADH (Table II). The two most active compounds are the octyl and nonyl whose effects are not statistically different from one another. These plants are shown at time of measurement (Figure 3) with the excised second internodes. C designates the control, A designates SADH (Alar), and the numbers indicate the number of carbons in the alkyl chain (R, Table I). No phytotoxicity was evident in any of the treatments.

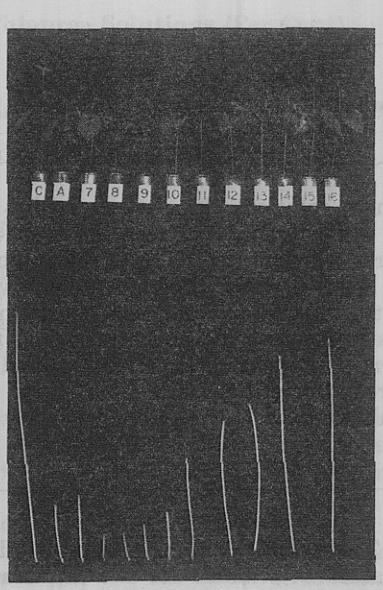


Figure 3. Effect of lanolin paste applications of quaternary ammonium compounds to Black Valentine beans: top, left to right: control (C), SADH (A), R = C_7, C_8, etc; bottom, excised second internodes in same order.

Duncan grapefruit seedlings treated with the octyl derivative were indistinguishable from the controls (Table III). The dodecyl compound gave highly significant retardation of new growth for a period of 3 weeks. At 4 weeks, its retardant effect was the same as that of the decyl compound, and both were significantly different from the control at the 5% level. After 2 weeks, the apices abscised in all ten seedlings sprayed with the dodecyl derivative. This was observed occasionally among the seedlings sprayed with the C_{14} and C_{16} compounds, but was not general throughout these treatments. After 4 weeks, those plants retarded showed accelerated growth compared to the controls until, at 10 weeks from the treatment date, all plants were of a uniform height.

Some phytotoxic symptoms were observed on grapefruit seedlings which appeared to increase with the length of the alkyl chain (R). These ranged from slight necrotic spots on the new, not fully expanded leaves treated with the dodecyl compound to general necrotic spots on all new growth, gumming on the stem and the leaf axis, and downward curling of new leaves treated with the hexadecyl derivative. However, fully expanded leaves exhibited no phytotoxic symptoms in any treatments.

CONCLUSION

Quaternary ammonium derivatives of 2-dimethylaminocyclohexanol represent a new and unique group of plant regulators having their own particular structural requirements for biological activity. Most significant is the fact that they are nonterpenoid and nevertheless much more active in all tests than the analogous compounds prepared from (+)-limonene. Although the growth regulant activity of these derivatives varies somewhat depending on the test plants used, maximum activity is exhibited by those compounds having 8–12 carbons in the alkyl chain attached to nitrogen. The cyclohexane ring having a quaternary ammonium substituent and a hydroxyl group on adjacent carbons comprises the structure which imparts plant growth regulant activity.

Table III. Growth Retardant Activity of Five Quaternary Ammonium Derivatives of 2-Dimethylaminocyclohexanol on Duncan Grapefruit Seedlings

Treatment	Total mean growth[a] (mm) at no. of weeks			
	1	2	3	4
Control	77c[d]	86c	86c	89b
Octyl[b]	72c	77c	77c	82b
Decyl	23ab	21ab	25ab	36a
Dodecyl	13a	5a[c]	9a	37a
Tetradecyl	49bc	50bc	53bc	69ab
Hexadecyl	48bc	52bc	52bc	80ab

[a] Ten replicates. [b] R attached to nitrogen (Table I). [c] Decrease in height due to apical abscission in all ten replicates. [d] Mean separation, within columns, by Duncan's multiple range test at the 5% level.

LITERATURE CITED

Biggs, R. H., University of Florida, unpublished results, 1973.

De Benneville, P. L., in "Medicinal Chemistry," Vol. III, Chapter 2, Blick, F. F., Cox, R. H., Ed., Wiley, Chapman and Hall, London, 1956.

Knight, B. E. A., Taylor, H. F., Wain, R. L., *Ann. Appl. Biol.* **63**, 211 (1969).

Krewson, C. F., Wood, J. W., Wolfe, W., *J. Agr. Food Chem.* **7**, 264 (1959).

Mitchell, J. W., Livingston, G. A., United States Department of Agriculture, Agriculture Handbook No. 336, Superintendent of Documents, U. S. Government Printing Office, Washington, D. C., 1968.

Newhall, W. F., *J. Agr. Food Chem.* **19**, 294 (1971).

Newhall, W. F., *HortScience* **7**, 254 (1972).

Newhall, W. F., Pieringer, A. P., *J. Agr. Food Chem.* **14**, 23 (1966).

Newhall, W. F., Pieringer, A. P., *J. Agr. Food Chem.* **17**, 153 (1969).

Pieringer, A. P., Newhall, W. F., *J. Agr. Food Chem.* **16**, 523 (1968).

Shapiro, S. L., Soloway, H., Shapiro, H. J., Freedman, L., *J. Amer. Chem. Soc.* **81**, 3993 (1959).

Steel, R. D. G., Torrie, J. H., "Principles and Procedures of Statistics," McGraw-Hill Book Co., New York, N. Y., 1960, p 481.

Tolbert, N. E., *J. Biol. Chem.* **235**, 475 (1959).

Winternitz, F., Mousseron, M., Canet, M., *Bull. Stê. Chim. Biol.* **33**, 369 (1951).

Received for review September 24, 1973. Accepted December 3, 1973. Florida Agricultural Experiment Stations Journal Series No. 4934.